State University of Makassar

INTERNATIONAL CONFERENCE ON MATHEMATICS, SCIENCE, TECHNOLOGY, EDUCATION AND THEIR APPLICATIONS

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SCREENING OF TEMBELEKANG PLANT (*LANTANACAMARALINN*) ACTIVE COMPOUNDS FORPREVENTION OF INFECTIOUS DISEASESINSKINWOUNDS

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Abstract

Lantana camaraLinn Plant is a plant that was developed as a potential source of secondary metabolites, which is, have potential as a drug based on ethnobotany approach. These plants are used especially as wound curein the South Sulawesi. It was believed to cure very quickly in various types of skin wounds.In the previous study, the results of Chloroformextractofthe*L. camaraLinn*leafshowedhighactivityagainstS.aureusandE.Colibacteria which eachinhibition zone of17.2mmand31.7mm. Buttime to heal of *L. camaraLinn*extract slower thanpatenteddrugsareoftenused. Therefore, the extraxt need toscreeningtoeliminateinactivecompounds.Theextractwas screened through several methods such as fractionation, purification and identification. Fractionation was done by flash column chromatography, purification by recrystallization and identification using color test, TLC and spectroscopic tests. The results showed therearethreecrystalsthat have beenscreenedinthe B fraction. These compounds react positively with Liberman-Buchard and $FeCl_3$ reagent and showed orange color solution and a brown precipitate that identify as terpenoids and flavonoid group. This is supported by the data from IR spectrum of the compound.

Keywords: Lantanacamara Linn leave; Plaster; Screening; Skin wounds.

1. Introduction

Indonesian society has made a series of efforts to prevention the disease using natural ingredients from a traditional medicine.Many people assume that the use of traditional medicine is relatively safer than synthetic or manufactured drugs.However, it does not mean traditional medicine does not have adverse side effects.This requires a more in-depth study on the chemical ingredients of natural materials and evidence of clinical efficacy for the use of traditional medicine is not only based on experience alone, but can be accounted for efficacy and safety are supported by scientific data.

Various types of plants with various medicinal purposes has been used as an alternative treatment for people who later supported by research from various agencies.Some plants that have beenpopular as are ginger as a cure hepatitis and enteritis, java tea as a diuretic, turmeric as an antiseptic, arthritis and hepatitis.

Tembelekang plant (*L. camara Linn*)is a potent plant and very strategic was developed as a formula resource and tracking of secondary metabolites that have the potential as a drug. These herbs can be easily found in all regions, especially South Sulawesi.This plant in South Sulawesi is used as a cure wounds and is believed to cure various types of skin wound infection very quickly.Thus, this plant has allegedly metabolism of secondary metabolites as a potential antibacterial, so it can be developed for antibiotics and accelerate healing of wound infection.

Accordingly, infection of the wound still continues to require the development and disbursement formula to find a way of healing that effectively and efficiently due to antibiotic resistance.Moreover, the raw material for making antibiotics is still very expensive because the majority materials are still imported.Thus, treatment with local materials that are cheaper, easily available and do not harm needs to be developed, such as *L. Camara Linn* plants.

Based on these descriptions, standardize of extracts and screening of secondary metabolites in plants *L.Camara Linn* for development as potential drugs as antibiotics on wound infection needs to be done carefully and thoroughly.Moreover, the roots of this plant has been proven efficacious as an anti-fungal (ShaukatSS *et.al*, 2002) and vegetable oil content efficacious as antibacterial (Sonibare OO and Effiong I, 2008).

End of the study are expected to be obtained the standard procedure in screening *of L.camaraLinn*leaf extracts, extract parameters for formulas as performed antiseptics in wound healing and prevent wound infection an effective, efficient and more practical.

2. Experimental

2.1 Instrumentation

The instrumentation used in this study is a glass equipment, evaporators, vacuum chromatography column, column flash chromatography, Buchner filter, maceration vessel, UV light (wavelength 254 nm and 365 nm), hot plate, pipette, analytical balance, oven, vials bottles, capillary tube, stirrer rods, melting point gauges, and IR spectrophotometer Shimadzu Prestige-21.

2.2Materials

The materials used are the leaf of *L.camara Linn* plants, some technical of organic solvents such as n-hexane, ethyl acetate, methanol, chloroform, and p solvents such as chloroform and ace several reagents such as Lieber Burchard reagent, Wagner, Drage $FeCl_3$ 1%, whatman filter paper, alum foil, $CeSO_4$ 2%, tissue, silica aluminum-coated TLC plates of silica G 60 F_{254}.

3. Procedure

Research methods will include sam preparation, isolation of secon metabolites *of L.camara Linn,* the eluent determination by TLC, fractio of extract by the best eluent use of col chromatography, monitoring analysis fractions with the highest number of sp and separated by TLC to obtain frac with the least number of spots, and purified by column chromatography TLC to obtain pu compound,Furthermore, the identific of pure compounds with FTIR.

3.1 Sample preparation

Sampling was carried out based on location of plants *L.Camara* almost fro throughout the district in So Sulawesi.Furthermore, leaf sam *L.camara* Linn was cleaned and then dri in the open air.The dried samples were into small pieces and pulverized using blender.

3.2 Extraction of secon ary metabolite are compounds of*L.camara Linn*

A total of 2.5 kg of *L.camaraLinn*leaf p powdered were macerated for 1 × 24 ho as much as five times consecutively wi chloroform.The extract was concentra by evaporation so obtained visco extract.Then put into dark bottles a stored in the refrigerator for analysis at later stage.

3.3 Fractionationwith column chromatography

fractionation stage begins with the vacuum column chromatographic a technique (VCC) with a suitable solvent ratio is n-hexane and ethyl acetate (8:2).13.48 grams chloroform extract was taken for VCC.Furthermore, the fractions of VCC were analyzed using thin layer chromatography (TLC) and combined based on similarity and stain patterns on the chromatogram.

4. Result and Discussion

4.1 Sample preparation and extraction

In testing the antibacterial activity of *L.camara Linn*leafshowed that chloroform extract has a high zone of inhibition against both gram positive and negative bacteria, namely *S.aureus* and *E.coli* (Dini I.*et.al*, 2010) and also refers to that used by the South Sulawesi community for the treatment of wounds is the leaf of the *L.camara Linn*plant. Hence in this study were selected as a sample of the leaf.

Maceration processto extractsecondary metabolitescompoundscontained in thesample.Maceration carried out for 1 × 24 hours as much as five times that aims to maximize the maceration process to extract natural ingredient compounds contained in the sample. Furthermore, the extract was concentrated with anevaporator to evaporate the solvent to obtain a chloroform extract of thick greenish-black with weighs 58.53 grams.

4.2 Fractionation ofextractand antibacterial activity comparison

Fractionation is done to separate the compounds contained in the sample. Chloroform extract obtained was fractionated by VCC.Prior to the VCC, first extract was analyzed by TLC to determine a suitable eluent that can be used when making VCC.Based on the TLC results were done with several comparison eluent is obtained that shows the chromatogram separation stain or pattern that is both eluent n-hexane:ethyl acetate at a ratio of 8:2.

Fractionation performed with eluent with enhanced polarity starts from 100% n-hexane, n-hexane: ethyl acetate at a ratio of 19:1 to 1:19, ethyl acetate 100%, up to 100% methanol. The results obtained by VCC as many as 23 fractions.

Based on further analysis by TLC of the similarity obtained stain patterns on the chromatogram.VCC fractions results are merged to obtain six fractions combined as in Table 1.

Table 1. Combined Fraction of Fractionation VCCResults

Fraction	Combined Fraction	Weight (gr)
2-6	A	3.50
7-9	B	5.70
10-14	C	5.40
15-16	D	4.40
17-20	E	4.80
21-23	F	5.09

Results of previous studies indicate that the drug patent healed faster 3-4 days than chloroform extract of L.*camara Linn* (Muharram *et.al*, 2010).Therefore, it will be screening the active compounds in the extract to eliminate inactive compound

Dini I *et al.* (2010) have carried out antibacterial test on fraction B and C by

examining the antibacterial activity against *S. aureus* and *E. coli* bacteria.The test results of the combined fractions are diameter of inhibition zone for each bacterium of *S. aureus* and *E.coli* in fraction B (18.3 mm and 12.4 mm) and fraction C (16.7 mm and 12.1 mm).And also the test results with the same bacteria on *L.camara Linn* leaf chloroform extract showed inhibition zone for bacteria *S. aureus* and *E.coli* in a row is 17.2 mm and 31.7 mm (Muharram *et.al*, 2010).

Comparison of the antibacterial activity results prove that there are certain compounds in the chloroform extract can inhibit the bacteria *S. aureus* and screening should be carried out first on all the fractions in order to obtain the active compounds to inhibit the bacteria *E.coli.*

4.3 Screening and Identification of Compounds

Screening of the B combined fractionthrough three stages of fractionation, purification, and identification.Fractionations were performed using press column chromatography techniques.But prior to first find a suitable solvent for use in the column using TLC technique.The results of several replications and variations of solvent ratio is obtained that a good solvent to be used is n-hexane:ethyl acetate (9:1).Fractionation of B fraction (2.60 g) by press column chromatography produces 119 fractions.Thenfollowed by TLC analysis with solvent ratio of n-hexane:ethyl acetate (9:1) and the combined fractionsobtained 8 fractions are B1 fraction (fractions 1-9);B2 (fractions 10-14);B3 (fractions 15-22);B4 (fractions 23-30);B5 (fractions 31-56);B6 (fractions 57-80);B7 (fractions 81-108);and B8 (fractions 109-119).

Fractionation results showed only B4 and B8 fraction that form of crystals after the solvent was evaporated.Hence, fractions of B4 and B8 respectively performed recrystallization with chloroform and ethyl acetate, and then analyzed by TLC with the hexane:ethylacetateeluent (9: 1) and obtained a single stain.Compounds in B4 fraction as compound of 1 in the form of needle-shaped crystals and B8 fraction as compound of 2 is irregular crystalline of greenish white flakes.

Identification of B4 and B8fraction aimed to determine the characteristics of theobtained compound .Identification of both is doneby including:

a. Specific reagents test of secondary metabolites group using reagent of FeCl3, LiebarmannBurchard, Wagner and Dragendorf.Compound of 1 positive on LiebarmannBurchard reagent with the formation of solution color changes to red orange.Compoundof 2 showed a positive reaction to the reagent $FeCl_3$with the formation color change of the test solution to yellow brown.
b. Spectroscopic measurements of compounds intended as the data in structure elucidation. Spectroscopic measurements that have been carried out for compounds of 1 and 2 are FTIR spectroscopy. Figures 1 and 2 show the FTIR spectra for each of the compounds.

Compoundsof 1 is the class of triterpenoids, which c ntained carbonyl groups are shown in IR absorption at wave 1690.63 cm^{-1},aliphatic C-H at 2947 cm^{-1}.

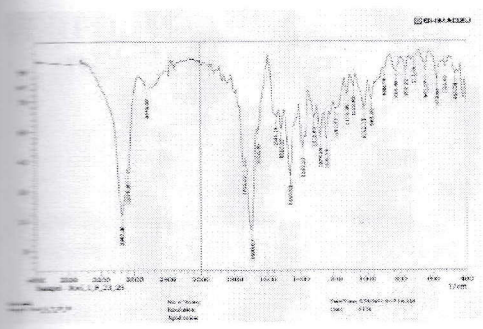

Figure 1. FTIR spectra of compound of 1

The FTIR chromatograms shows compound of 1functional group as seen from the respective absorption peaks at wave numbers that are identified as follows:

Table 2. Compound of 1 functional groups interpretation

No.	Wavenumber (cm^{-1})	Functional groups
1.	2947.36	C-H
2.	1690.63	C=O
3.	1459.33	-CH2-
4.	1382.23	-CH3

Figure 2 shows the FTIR chromatogram of functional groups compound of 2 as seen from the respective absorption peaks at wave numbers that are identified as follows:

Table 3. Compound of 2 functional groups interpretation

No.	Wave number (cm^{-1})	Functional groups
1.	3278.89	-OH
2.	2947.36	-CH
3.	1706.05	C=O
4.	1467.04	-CH2-

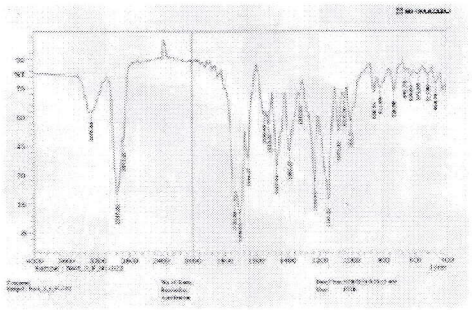

Figure 2. FTIR spectra of compound of 2

The compound of 2 wasflavonoid compounds containing hydroxyl group at wave number of 3278.89 cm-1, carbonyl at 2947.36 cm-1 ,and aromatic at 1513.30 cm-1 wave number.

The FTIR spectroscopy measurements have not been sufficient for the elucidation of the structure, so it will be another spectroscopic measurements to determine the structure of compounds. And also measuring the bioactivity of the combined fractions that would otherwise be performed for screeningsecondary metabolites in order to obtain the active compounds in preventing infection in skin wounds.

5. Conclusion

The results of screening by fractionation and purification techniques discovered class of terpenoids and flavonoids compounds through the identification of FTIR spectroscopy. However, the need to test the bioactivity for comparison with the chloroform extract of the leaf*of L.camara Linn.*

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CERTIFICATE

Awarded to

Pince Salempa

as

PRESENTER

(ISOLATION AND STRUCTURE ELUCIDATION OF B-SITOSTEROL COMPOUND FROM THE N-HEXANE FRACTION OF ROOT WOOD OF *PTEROSPERMUM SUBPELTATUM* C.B. ROB)

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Rector

Prof. Dr. H. Arismunandar, M.Pd.

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